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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
UTILIZATION RESEARCH

LIST OF PUBLICATIONS AND PATENTS

of the
Northern Utilization Research Branch
Peoria, Illinois

July - December 1954

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PUBLICATIONS

[Publications marked (*) are not available for distribution]

ANALYTICAL TOTAL ACID HYDROLYSIS OF DEXTRANS.

R. J. Dimler, H. A. Davis, G. J. Gill, and C. E. Rist.
Analyt. Chem. 26(7): 1142-1146. July 1954.

The hydrolysis of dextran and destruction of D-glucose, under a limited range of acid hydrolysis conditions, were studied to provide a basis for selection of optimum conditions for complete acid hydrolysis of dextrans, either for determining constituent monosaccharide units or for measuring the concentration of dextran solutions. Hydrochloric acid and sulfuric acid were shown to be essentially interchangeable provided the normality of sulfuric acid used was about twice that of the hydrochloric. A standardized procedure is given and its applicability to dextrans of different structures and properties, as well as to starch, is demonstrated.

EFFECT OF ALCOHOLS ON HYDROCARBON AUTOXIDATION.

Charles F. Frye, Carl B. Kretschmer, and Richard Wiebe.
Indus. and Engin. Chem. 46(7): 1517-1520. July 1954.

In connection with work on the use of alcohol in motor fuels, oxidation rates of four hydrocarbons were measured in the presence and absence of primary, secondary, and tertiary alcohols. It was found that alcohols accelerate the oxidation of tetralin and cyclohexene, but retard the oxidation of di-isobutylene and octene-1. The oxidation of mixtures of di-isobutylene with tetralin and cyclohexene was also retarded in the presence of alcohol.

The results are explained in terms of a mechanism in which alcohol molecules participate in the oxidation chain reaction. Depending on the relative rates of the various steps, the mechanism can lead to either acceleration or retardation of the oxidation by alcohol, as observed experimentally.

FERMENTOR FOR SMALL-SCALE SUBMERGED FERMENTATIONS.
Robert G. Dworschack, Adolph A. Lagoda, and Richard W. Jackson.
Appl. Microbiol. 2(4): 190-197. July 1954.

The description and operation of a 20-liter stainless-steel fermentor similar in design to industrial units are given. The accessories, such as aerators, agitators, antifoam system and electrode, and equipment to facilitate the continuous manual measurement and control of pH during the fermentation are also described.

Three comparisons of data obtained from the 20-liter and a larger pilot-plant fermentor are given for the production of dextransucrase, calcium gluconate, and riboflavin.

SOYBEAN "LECITHIN" AND ITS FRACTIONS AS METAL-INACTIVATING AGENTS.
C. D. Evans, Patricia M. Cooney, C. R. Scholfield, and H. J. Dutton.
Jour. Amer. Oil Chem. Soc. 31(7): 295-297. July 1954.

Unfractionated soybean lecithin is effective as a metal-inactivating agent, but has defects in color and flavor when used at levels required for optimal oxidation stability. The darkening of lecithin-treated salad oils during deodorization is associated with the presence of phosphatides containing amino groups. An inositol-containing phosphatide and phosphatidyl choline which contain no amino groups are relatively color stable. However, the maximum level at which these color-stable phosphatides may be added is limited by their tendency to cause undesirable flavors upon storage. Sugar constituents apparently play no significant part in color, flavor, or oxidative stability of soybean lecithin.

AQUEOUS VAPOR PRESSURE OF SOYBEAN MEAL AND ITS FRACTIONS.
P. A. Belter, C. R. Lancaster, and A. K. Smith.
Jour. Amer. Oil Chem. Soc. 31(9): 388-392. September 1954.

The aqueous vapor pressure of denatured and undenatured soybean oil meals and fractions thereof have been measured by a manometric technique. The determinations were conducted at temperatures of 30, 40, 50, 60, and 80° C. for moisture contents ranging from 3-1/2 to 32 percent moisture. The results were plotted as isoteric lines versus temperature and used for obtaining the variation of the ratio of heat of desorption to latent heat of vaporization for various moisture contents. This ratio was significantly increased above 1.0 at moisture contents below 7 percent for the soybean oil meals and the water-soluble fraction (pH 7.4) and at moisture contents below 12 percent for the water-soluble fraction at pH 4.5 (soybean whey).

DETERMINATION OF DEXTRAN STRUCTURE BY PERIODATE OXIDATION TECHNIQUES.

John W. Sloan, B. H. Alexander, R. L. Lohmar, I. A. Wolff, and C. E. Rist.
Jour. Amer. Chem. Soc. 76(17): 4429-4434. September 5, 1954.

The determination of the structure of dextrans produced by several strains of *Leuconostoc mesenteroides* by the method of periodate oxidation, hydrogenation of the dialdehyde, hydrolysis of the polyol, and chromatographic assay of the fragments is described. A new method was devised for isolation of oxidized dextrans in good yields for use in characterization. Five percent 1,3-linked anhydroglucose units were shown to be present in dextran from *L. mesenteroides* NRRL B-512 and 3 percent in dextran from *L. mesenteroides* NRRL B-1064. The presence of 1,6-linkage in the amounts predicted by other methods was generally confirmed, but 1,4-linkage appeared in less than the expected quantity.

EVALUATION OF THE PERIODATE OXIDATION METHOD FOR STRUCTURAL ANALYSIS OF DEXTRANS.

John C. Rankin and Allene Jeanes.
Jour. Amer. Chem. Soc. 76(17): 4435-4441. September 5, 1954.

Specific structural data have been obtained on a very large number of dextrans by the method of oxidation with sodium metaperiodate. From the analytical results, the type and proportion of linkages between the glucose units were calculated.

The accuracy and precision of the analytical results have been established. The method is applicable to structural analysis of other polysaccharides.

THE STRUCTURE OF ALPHA-AMYLASE MODIFIED WAXY-CORN STARCH.

R. L. Lohmar.
Jour. Amer. Chem. Soc. 76(18): 4608-4611. September 20, 1954.

Graded hydrolysis of waxy-corn starch by malt-α-amylase produced a series of modified amylopectins which were characterized by their reducing power, the amount of formic acid produced on periodate oxidation, and their convertibility by β-amylase. From these determinations, the average chain length and the average inner- and outer-branch lengths were calculated. These calculated values decreased as the extent of hydrolysis progressed. Hence, the modified products are best represented as polymers differing from the parent amylopectin in molecular weight and in the average length of both inner and outer branches. It is concluded further that the initial hydrolysis by malt α-amylase is not confined to the outer branches, but that the longer inner branches are attacked simultaneously. Similar studies on modified β-limit dextrins confirmed this conclusion.

FLASH DESOLVENTIZING.
P. A. Belter, O. L. Brekke, G. E. Walther, and A. K. Smith.
Jour. Amer. Oil Chem. Soc. 31(10): 401-403. October 1954.

A new flash desolventizing process based on fluidization principles is described. It has been shown in this investigation that commercial hexane can be removed from soybean flakes in several seconds without excessive solvent loss and with a minimum of protein denaturation. Other advantages of this system are discussed. This unit is recommended for existing plants as an auxiliary desolventizer for expansion of extraction facilities or to furnish products of high protein solubility as the demand for them occurs.

*PRINCIPLES AND PRACTICE OF ALCOHOL-WATER INJECTION (published as DIE ALKOHOL/WASSER-EINSPRITZUNG IN OTTOMOTOREN).
Richard Wiebe.
Erdol und Kohle 7: 643-647. October 1954.

Principles of alcohol-water injection are explained and examples shown to indicate the adaptability of injection to the operation of farm tractors and military trucks.

RESEARCH DEVELOPMENTS AT THE NORTHERN UTILIZATION RESEARCH BRANCH.
J. C. Cowan.
Soybean Digest 14(12): 14-16. October 1954.

A review is presented of investigations on soybean phosphatides, their fractionation, isolation, and composition. The relationship of the browning reaction to the color and processing of soybean lecithin is described.

RECENT PROGRESS IN STUDIES OF BREAD STALING.
M. M. MacMasters and P. D. Baird.
Bakers Digest 28(5): 92-95, 103. October 1954.

This review presents a summary of the more important papers on bread staling and related topics that have appeared in the scientific literature during the period 1949-1954. Topics discussed include the changes which constitute staling, objective tests for staling, the relation of starch to changes in the crumb, the role of gluten in staling, changes in flavor and odor of bread during staling, the use of adjuncts to control crumb firming, and physical methods of controlling crumb firming. A bibliography of 54 references is included.

SYNTHETIC LUBRICANTS FROM POLYHYDROXYSTEARIC ACIDS.
L. E. Gast, C. B. Croston, W. J. Schneider, and H. M. Teeter.
Indus. and Engin. Chem. 46(10): 2205-2208. October 1954.

A series of derivatives of dihydroxystearic acid has been prepared and some of the viscometric properties that are of interest in evaluating these derivatives as synthetic lubricants have been determined. Pour points for these compounds were mostly in the range of -60° to -70° F. Viscosities at 100° F. varied from 15 to 30 cs.; however, at -40° viscosities were of the order of 4,000 cs. or greater.

THERMODYNAMICS OF ALCOHOL-HYDROCARBON MIXTURES.

C. B. Kretschmer and R. Wiebe.
Jour. Chem. Phys. 22(10): 1697-1701. October 1954.

The thermodynamic properties of alcohol-hydrocarbon mixtures are explained on the basis of association of the alcohol to form polymers of all orders from two to infinity. Our treatment differs from that of Redlich and Kister in using the Flory-Huggins expression for free energy, and an equilibrium constant in terms of concentrations rather than mole fractions. The equations derived are in satisfactory agreement with vapor-pressure data for several systems. They also agree with the experimental energy of mixing and excess entropy for ethanol-methylcyclohexane solutions. For mixtures of alcohols with aromatic hydrocarbons, it is necessary to assume that each alcohol monomer or polymer can combine with one molecule of hydrocarbon.

CHARACTERIZATION AND CLASSIFICATION OF DEXTRANS FROM 96 STRAINS OF BACTERIA.

Allene Jeanes, W. C. Haynes, C. A. Wilham, J. C. Rankin, E. H. Melvin, Marjorie J. Austin, J. E. Cluskey, B. E. Fisher, H. M. Tsuchiya, and C. E. Rist.
Jour. Amer. Chem. Soc. 76(20): 5041-5052. October 20, 1954.

Heretofore, relatively few of the dextrans potentially available through bacterial fermentation have been prepared and characterized. The rapidly increasing significance of these polyglucosans for medical, industrial, and research purposes motivated a survey of the types obtainable. We have prepared dextrans of high purity from 96 individual strains and characterized them by periodate-oxidation-reaction analysis; by measurement of optical rotation, intrinsic viscosity, the concentration-dependent parameter of viscosity, and infrared absorption; and by observations on solubility and gum properties. On the basis of the types and proportions of glucosidic linkages present as indicated by periodate-oxidation-reaction analysis, the dextrans have been grouped into three classes. Six dextrans could not be placed in any class because they have been separated into two or more components belonging in different classes. The dextrans prepared might not yet represent all possible classes, and reclassification of some of them might be indicated when more specific structural analyses become available.

USEFUL CRITERIA FOR SPECIES DIFFERENTIATION IN THE GENUS *STREPTOMYCES*.
C. W. Hesseltine, R. G. Benedict, and T. G. Pridham.
Ann. N. Y. Acad. Sci. 60(1): 136-151. October 29, 1954.

Taxonomic methods employed in species characterization of *Streptomyces* have been reviewed and evaluated. Need for uniformity in methods of study and reporting of data has been emphasized. Methods which appear to be of most importance in defining a species have been enumerated.

A key, based on morphology and spore color, is proposed for separation of the major groups in the genus. The use of physiological criteria in more precise delimitation of species is stressed.

Excellent correlation between microscopic characteristics, gross cultural appearance, and physiological activity was noted with the isolates studied.

ISOLATION AND UTILIZATION OF VEGETABLE PROTEINS.
Allan K. Smith.
Econ. Bot. 8(4): 291-315. October-December 1954.

The literature is reviewed on the isolation and properties of the vegetable proteins that have been proposed for industrial utilization. The properties required of a protein suitable for industrial purposes and the methods used for their separation are given. The proteins reviewed are from the seed of the flax, sunflower, castor, soybean, peanut, cottonseed, and corn.

THE BREAD STALING PROBLEM. MOLECULAR ORGANIZATION OF STARCH UPON AGING OF CONCENTRATED STARCH GELS AT VARIOUS MOISTURE LEVELS.
N. N. Hellman, Barbara Fairchild, and F. R. Senti.
Cereal Chem. 31(6): 495-505. November 1954.

Starch gels containing 29 percent to 63 percent water were prepared by heating starch of the desired water content for one-half hour at 96° C. Starch systems containing more than 43 percent water lost all crystallinity demonstrable by X-ray diffraction as a result of the heat treatment. Storage of the gels under conditions which prevented moisture loss permitted the starch to reorder and exhibit crystalline X-ray diffraction patterns. The moisture content at which the starch gels were aged determined the structural modification which appeared. Gels containing less than 29 percent water gave Type A diffraction patterns; between 32 percent and 39 percent water content, the gels developed mixed A and B diffraction patterns which also might be classified as C diffraction patterns. Above 43 percent water content, the gels developed typical B diffraction patterns. Rate of crystallinity development at different moisture contents differed; however, in all cases it was essentially completed in 8 days. Gels containing 56 percent or more water showed low rates of crystallization and decreased amounts of molecular organization. Reheating of aged samples caused loss primarily of B-type crystallinity of the crystallization process. The Type V diffraction pattern, described by Katz to be produced by freshly pasted starch gels, was shown to arise from a complex of starch with the fat normally present in wheat starch preparations.

A CLARIFICATION OF THE RELATIONSHIP OF CANDIDA GUILLIERMONDII TO OTHER YEASTS BY A STUDY OF THEIR MATING TYPES.

Lynferd J. Wickerham and Kermit A. Burton.
Jour. Bact. 68(5): 594-597. November 1954.

Some of the more common and best known species of yeasts previously classified in the nonascosporogenous genera *Torulopsis* and *Candida* are haploid yeasts which occur in nature as opposite sexes. When these sexes are brought together under favorable conditions they mate and produce ascospores. Yeasts which belong to the same species but which have been unknowingly classified as separate species can be shown to be identical when the mating types of the two supposed species mate, and their identity is further confirmed by similarity of their biochemical properties. Strains which have been placed in the same species but are actually different species may be recognized as such when the mating types of one species fail to mate with the mating types of the other species. Here again, differences in physiology and morphology confirm the fact that one name is being used to cover two species.

In this paper the taxonomy of four species of yeasts has been clarified by the above procedures.

ECONOMIC FACTORS TO BE CONSIDERED IN THE USE OF SUGARCANE BAGASSE AS A RAW MATERIAL FOR PAPER AND BOARD MANUFACTURE.

E. C. Lathrop.
U. S. Dept. Agri. ARS-71-2, 24 pp. (Processed). November 1954.

Variations in quality and quantity of bagasse due to sugarcane variety and culture and to cane harvesting and milling practices produce rather wide variations in the chemical and particularly in the physical characteristics of bagasse, including dirt and carbon contaminations. Surplus bagasse is present at most sugar mills. Problems concerning baling, handling, storage, losses during storage, and cost of stored bagasse are evidently not generally well understood.

There is as yet no method for the economic use of "total" bagasse. Pith is objectionable in paper manufacture. Methods of separating the pith from the fiber have been perfected. Depithed fiber is not more costly for use in papermaking. Pith itself may be used, particularly as an absorbent for molasses for feeding. The sugar mill is the logical place for preparation of depithed fiber.

Wider availability of paper made possible through use of bagasse will of itself have far-reaching effects in improving social and economic conditions in less developed countries. Integration of the sugar and paper industries may have even a more profound effect, if advantage is taken of all the potential opportunities such a relationship offers.

THE EFFECT OF POTASSIUM UPON THE GROWTH OF MICROCOCCUS PYOGENES.

W. C. Haynes, R. W. Kuehne, and L. J. Rhodes.
Appl. Microbiol. 2(6): 339-344. November 1954.

In a search for factors which would stimulate the growth of Micrococcus pyogenes, NRRL B-1317P, it was observed that doubling the concentration of the principal nutrient in the culture medium resulted in a higher cell population. Inasmuch as the single-strength medium was believed sufficient in both carbon and nitrogen to support greater cell crops than were obtained, a contaminating trace nutrient was suspected as the growth stimulant. Consideration of the medium composition suggested minerals as the more promising category for primary exploration. With this in mind, the medium was supplemented with Speakman salts, an inorganic mixture customarily included in microbiological assay media. This modification resulted in an approximate three-fold increase (to 15 billion) in the number of cells produced. Fractionation of the salt solution eventually revealed potassium as the element primarily responsible for the marked stimulation to growth. In further studies it was found that media containing less than about 0.0045 mM K^+ per ml. would not permit maximum growth, whether measured as number of viable cells or as packed cell volumes. Between 0.0045 and 0.05 mM K^+ per ml. the organism showed maximum response, but above this range the cell population decreased with increasing potassium.

Several other strains of M. pyogenes of both albus and aureus varieties responded to the presence of K^+ in a similar manner. Thus it is likely that all strains of this species will produce more cells and more cell substance in the presence of optimal levels of potassium.

FURTHER STUDIES ON CINNAMYCIN, A POLYPEPTIDE ANTIBIOTIC.

William Dvonch, Odette L. Shotwell, Robert G. Benedict, Thomas G. Pridham, and Lloyd A. Lindenfelser.
Antibiotics and Chemotherapy 4(11): 1135-1142. November 1954.

Cinnamycin hydrochloride was further purified by countercurrent distribution. Acid hydrolyzates yielded eight ninhydrin spots on paper chromatograms with Rf values corresponding to aspartic acid, arginine, glutamic acid, proline, phenyl alanine, valine, meso-lanthionine, and β-methyllanthionine. Cinnamycin was shown not to contain L-cystathionine or its optical isomers. The description of Streptomyces cinnamoneus has been completed.

POLYMERIZATION, COPOLYMERIZATION, AND ISOMERIZATION.
J. C. Cowan.
Jour. Amer. Oil Chem. Soc. 31(11): 529-535. November 1954.

This paper is a review of the recent work on polymerization, copolymerization, and isomerization of glycerides.

STUDIES ON THE EFFECT OF DRYING CONDITIONS UPON THE COMPOSITION AND SUITABILITY FOR WET MILLING OF ARTIFICIALLY DRIED CORN.
M. M. MacMasters, F. R. Earle, H. H. Hall, J. H. Ramser, and G. H. Dungan.
Cereal Chem. 31(6): 451-461. November 1954.

Ear corn harvested at 40, 30, and 20 percent moisture content, each, in 1950 and 1951 was divided into two lots of samples which were dried at low and high relative humidity, respectively, each sample being divided into subsamples which were dried at approximately 120°, 130°, 140°, and 150° F., respectively. Controls were air-dried at room temperature. High sugar content was maintained by artificial drying. In general, content of niacin, pantothenic acid, riboflavin, biotin, and pyridoxine was relatively unaffected by drying conditions. Data obtained by laboratory processing for starch gave no clear correlation with drying conditions, although corn dried at 150° F. tended to process poorly. Dissimilarities in results obtained with samples from the two crop years are attributed to differences in the chemical or physical state of kernel constituents, resulting from differences in growing seasons.

THE CONTRIBUTION THAT SUGARCANE BAGASSE CAN MAKE TO THE PAPER AND BOARD INDUSTRIES.
E. C. Lathrop and S. I. Aronovsky.
U. S. Dept. Agri. ARS-71-1, 22 pp. (Processed). November 1954.
TAPPI 37(12): 24A, 26A, 28A, 30A, 32A, 34A, 38A, 40A, 42A, 44A, 46A, 48A, 50A, 52A, 54A, 56A, 58A. December 1954.

Sugarcane bagasse is a valuable material for the pulp and paper industries, augmenting insufficient supplies of wood and other pulps. Usable papers and wallboards of low quality may be manufactured from pulps made from whole or screened bagasse. By pulping with pressure, Celdecor, or mechano-chemical processes, using chemicals composed of or containing caustic soda, much higher grade products including bleached pulps can be made. The best use for bagasse pulps will be as blends with virgin wood pulps, waste papers, and fillers.

Best results can be expected from pulping depithed bagasse fiber only. Use of the separated pith for fuel, feed, or paper filler would cut pulp cost. Depithed bagasse pulps can be used solely for manufacture of highest quality corrugating board medium and glassine and certain kinds of waxing papers. They can also be blended with kraft or sulfite softwood pulps to make superior wrappings, bag and multiwall bag papers, and liner boards.

Bleaching and blending permits the manufacture of very high-quality printing and specialty papers. In countries where newsprint is not available, a sheet can be made of 100 percent bagasse which might be accepted by the newspaper publisher. Standard quality newsprint can be manufactured from mechanical pulp and bleached bagasse fiber.

X-RAY AND PHOTOMICROGRAPHIC EXAMINATION OF RICE.

Joseph T. Hogan, R. A. Larkin, and M. M. MacMasters.
Jour. Agr. Food Chem. 2(24): 1235-1239. November 24, 1954.

X-ray techniques have been used successfully at the Southern Branch for the rapid examination of rough (unhulled) rice for cracks, checks, insect damage, and immature seed. Radiographic evidence of cracks (or checks) in grains of rough rice was confirmed at the Northern Branch by photomicrographs of halves of the same kernels. Improved techniques were used in the preparation of thin sections of rice kernels for study of the detail of the inner structure of the kernels. Quality factors, such as yields of head rice, color, stability to oxidation, and nutritive value, are related to the structural elements of the rice kernel that remain in the milled rice.

EFFECT OF AGITATION ON SELECTIVITY IN THE HYDROGENATION OF SOYBEAN OIL.

R. E. Beal and E. B. Lancaster.
Jour. Amer. Oil Chem. Soc. 31(12): 619-625. December 1954.

Experiments in hydrogenating soybean oil were conducted in the pilot plant in a baffled reaction vessel having a maximum capacity of 25 gallons. A simple turbine agitator was compared with a gas dispersing agitator over a range of temperature, pressure, catalyst concentration, batch size, and speed of agitation.

The gas dispersion agitator gave a more stable hydrogenated oil than the turbine type, but at some sacrifice in the speed of the reaction. Consideration of the results leads to a possible extension of the theory of selective hydrogenation.

PRODUCTION OF CLINICAL-TYPE DEXTRAN. II. PARTIAL ACID HYDROLYSIS AND FRACTIONATION OF THE DEXTRANS FROM LEUCONOSTOC MESENTEROIDES NRRL B-742 AND STREPTOBACTERIUM DEXTRANICUM NRRL B-1254.

I. A. Wolff, R. L. Mellies, R. L. Lohmar, N. N. Hellman, S. P. Rogovin, P. R. Watson, J. W. Sloan, B. T. Hofreiter, B. E. Fisher, and C. E. Rist.
Indus. and Engin. Chem. 46(12): 2605-2610. December 1954.

Clinical-type dextran fractions of the proper molecular weight and molecular weight distribution to meet current military specifications have been prepared from two dextrans differing in chemical structure from NRRL B-512 dextran. Conditions were established for partial hydrolysis with sulfuric acid, followed by fractional precipitation with ethanol, of each dextran. These laboratory procedures were applied in the pilot plant to obtain several pounds of clinical-type fraction from each dextran. The kinetics of hydrolysis and the effect of variables in the hydrolysis procedure on the yield and properties of such dextran fractions are discussed. The preferred procedure for hydrolysis and fractionation to obtain a clinical-type fraction is dependent on the structure of the dextran hydrolyzed. Hydrolysis of dextran has been found to involve other factors than random cleavage of equivalent bonds.

RESEARCH RELATED TO PROTECTIVE COATINGS AT THE UTILIZATION RESEARCH BRANCHES.

J. C. Cowan.
Official Digest of the Federation of Paint and Varnish Production Clubs 26(359): 1211-1218. December 1954.
Amer. Paint Jour. 39(13): 100, 102-103, 106, 108, 110, 112-113, 116. December 13, 1954.

Three accomplishments of research related to protective coatings at the Utilization Branches are described: Epoxidation, acetoglycerides, and distribution of fatty acids in linseed oil.

PATENTS

[These patents are assigned to the Secretary of Agriculture.
Copies of patents may be purchased from the
U. S. Patent Office, Washington, D. C.]

PRODUCTION OF DEXTRANSUCRASE.

Henry M. Tsuchiya and Harold J. Koepsell.
U. S. Patent 2,686,147. August 10, 1954.

Dextran is produced by first obtaining a dextran-synthesizing enzyme secreted by microorganisms, such as *Leuconostoc mesenteroides*, and then subjecting aqueous solutions of sucrose to the action of the secreted enzyme. Increased yields of the enzyme are realized by conducting the enzyme-producing step at a pH between 6.0 and 7.0.